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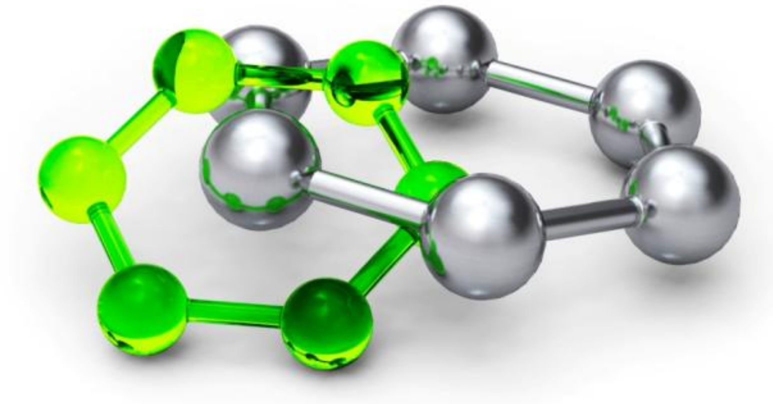
# RNA-seq+ - Analysis

**Vojtěch Bystrý**

**16. December 2019**



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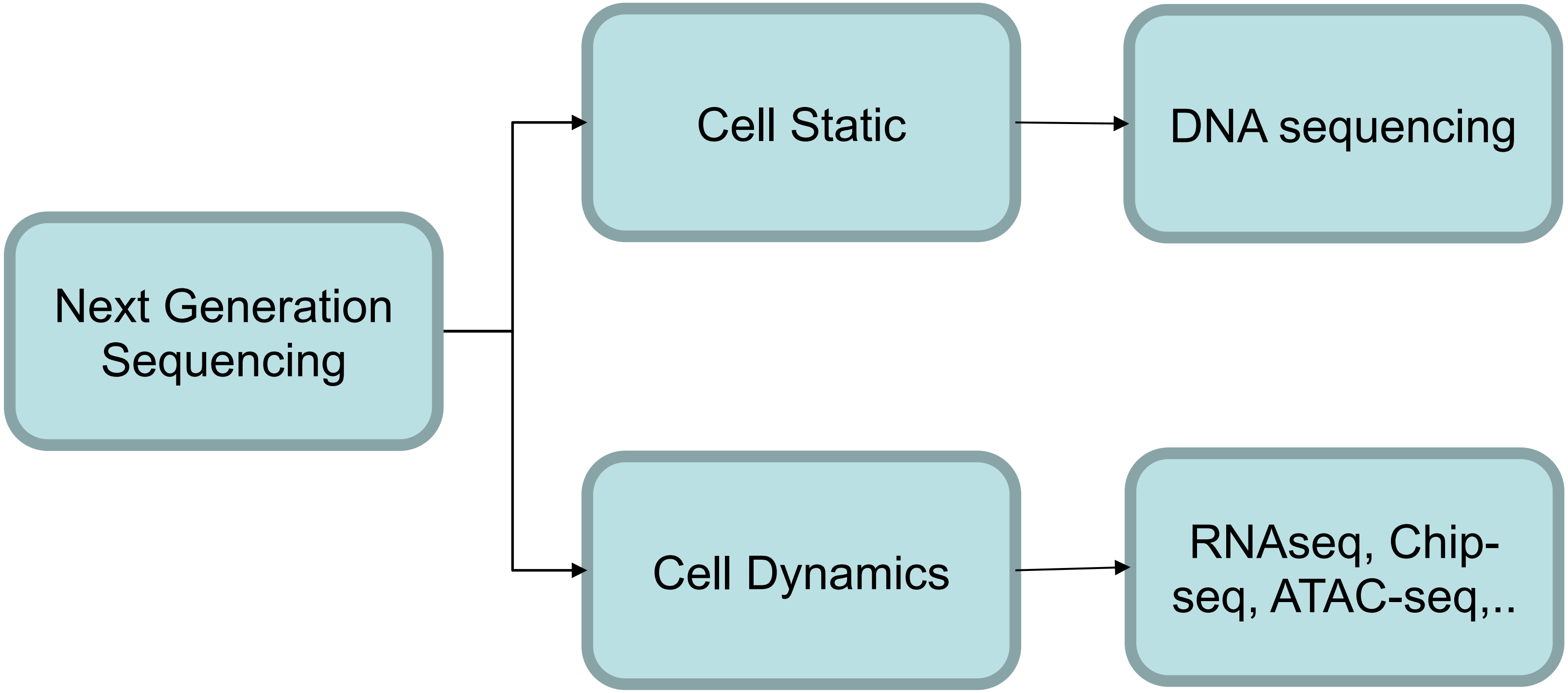
# NGS experiments



Next Generation Sequencing

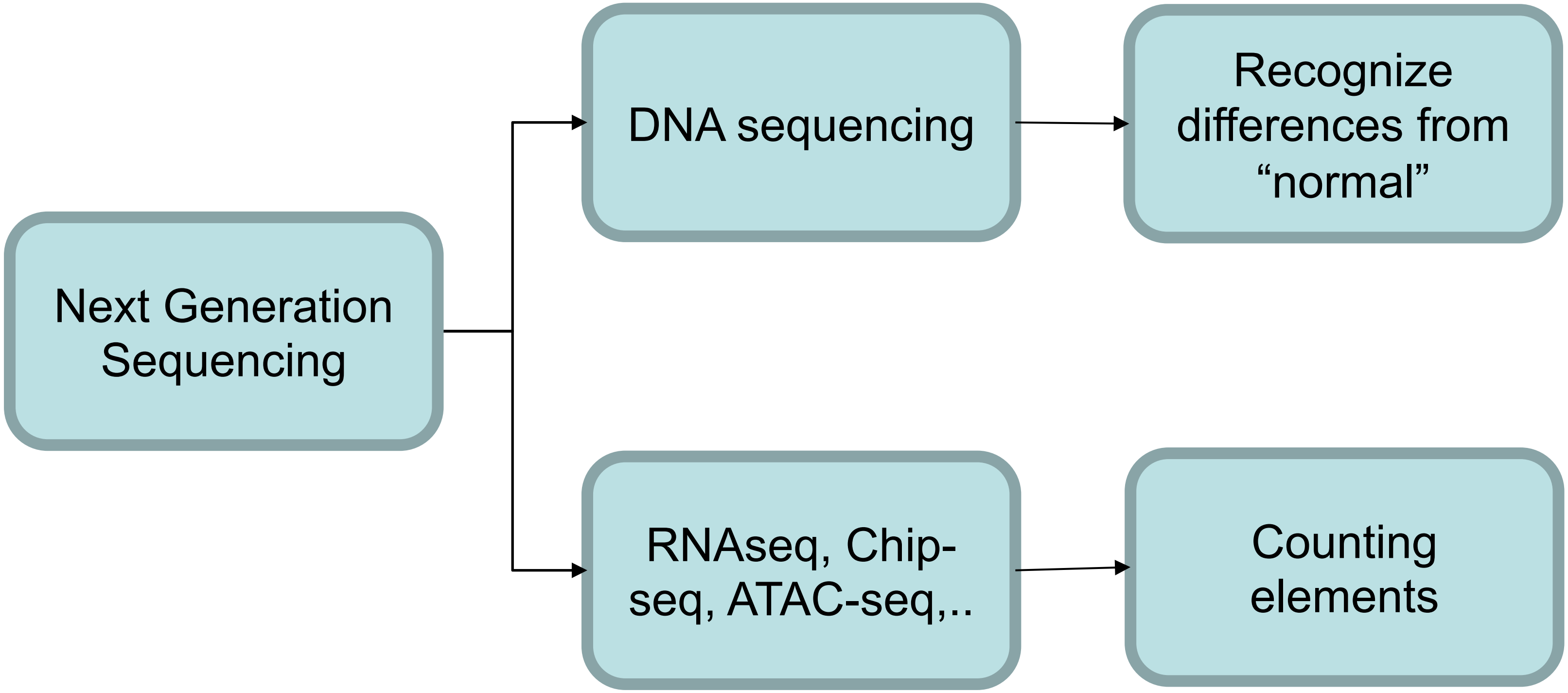
Cell Static

DNA sequencing

Cell Dynamics

RNAseq, Chip-seq, ATAC-seq,..

# NGS experiments



Next Generation Sequencing

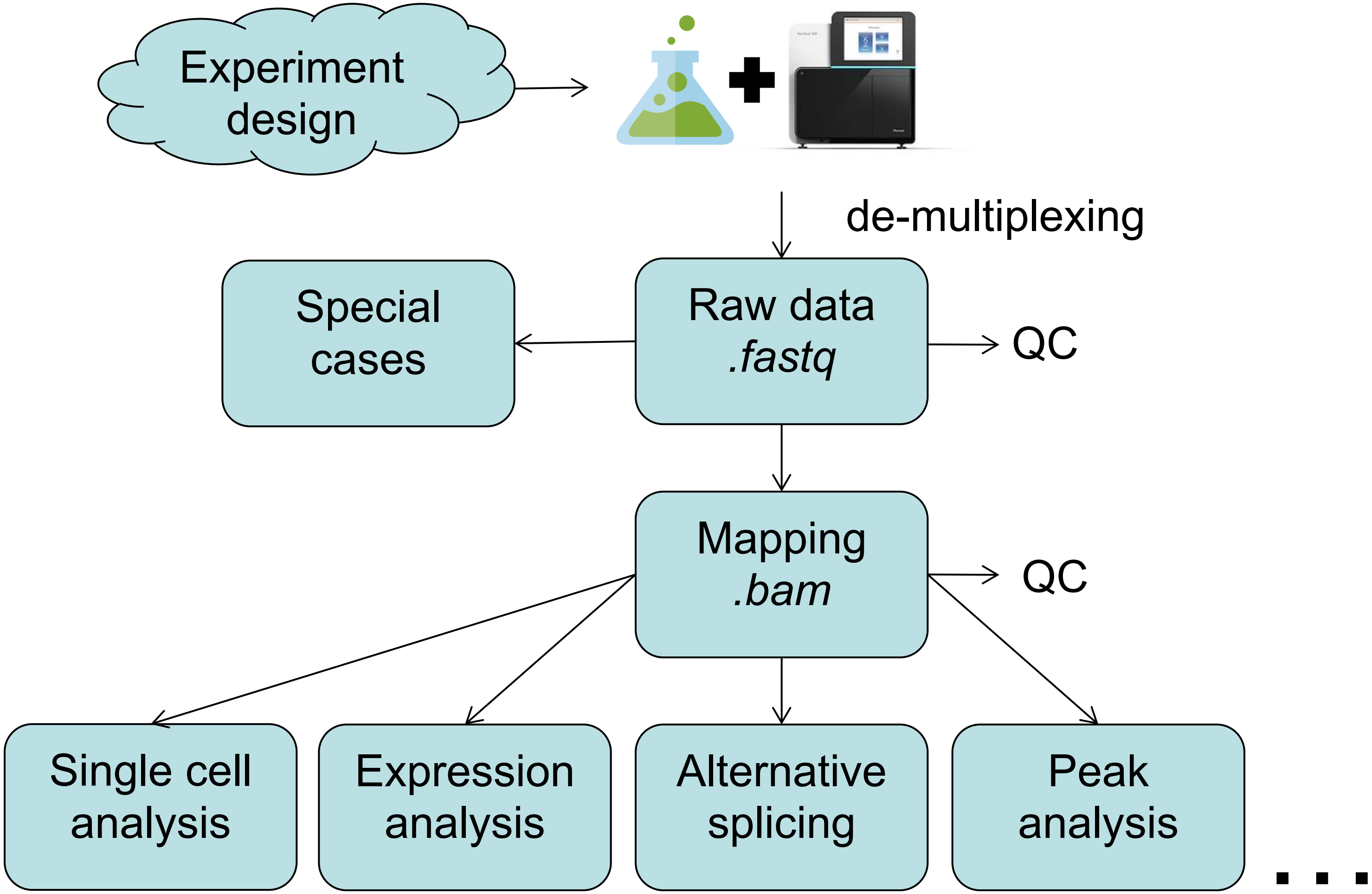
DNA sequencing

Recognize differences from “normal”

RNAseq, Chip-seq, ATAC-seq,..

Counting elements

# NGS data analysis workflow



Experiment design

de-multiplexing

Special cases

Raw data *.fastq*

QC

Mapping *.bam*

QC

Single cell analysis

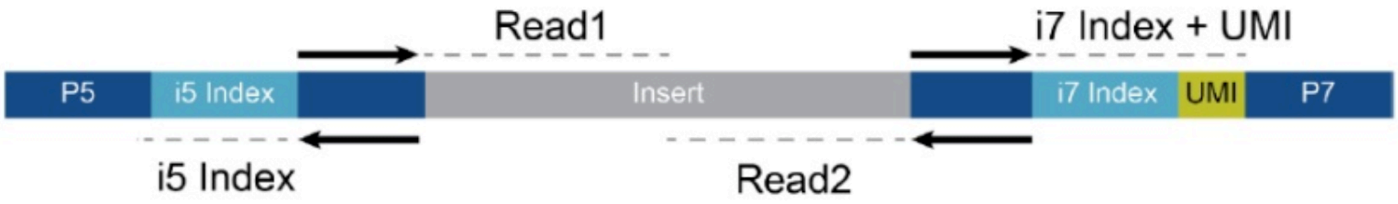
Expression analysis

Alternative splicing

Peak analysis

. . .

# UMI – unique molecular identifiers



- Each molecular fragment gets unique n-base sequence (n ~ 8-12)
- Usage:
  - Mark duplicates

# Raw data - QC

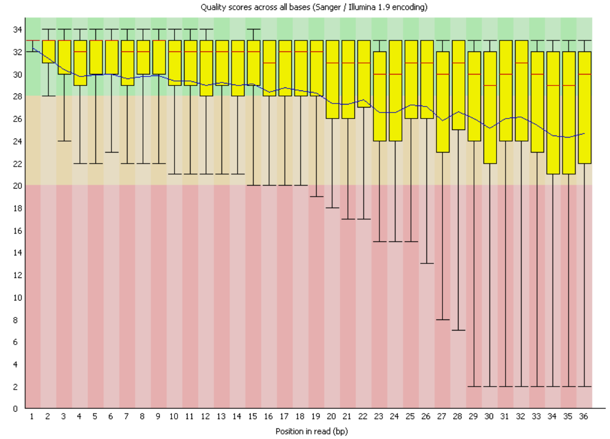
- **Fastq - q stands for quality – coded phred score**

```
CFFFFEFFGCEEGECFGGGGAFF87@E:++6C<++3:,8,33,,:,,,:,,,:,,,
```

$$Q = -10 \cdot \log_{10} P$$

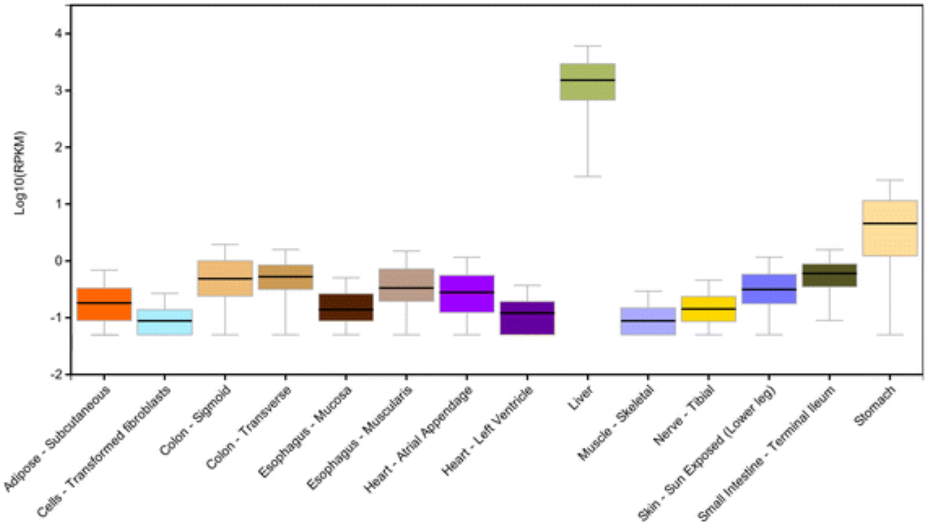
| Quality | Error probability |
|---|---|
| 5 | 31% |
| 10 | 10% |
| 20 | 1% |
| 30 | 0.1% |

- **Very good for early problem detection**
- **Reasonable for trimming and read filtering**
  - **RNA seq - above phred score 5**



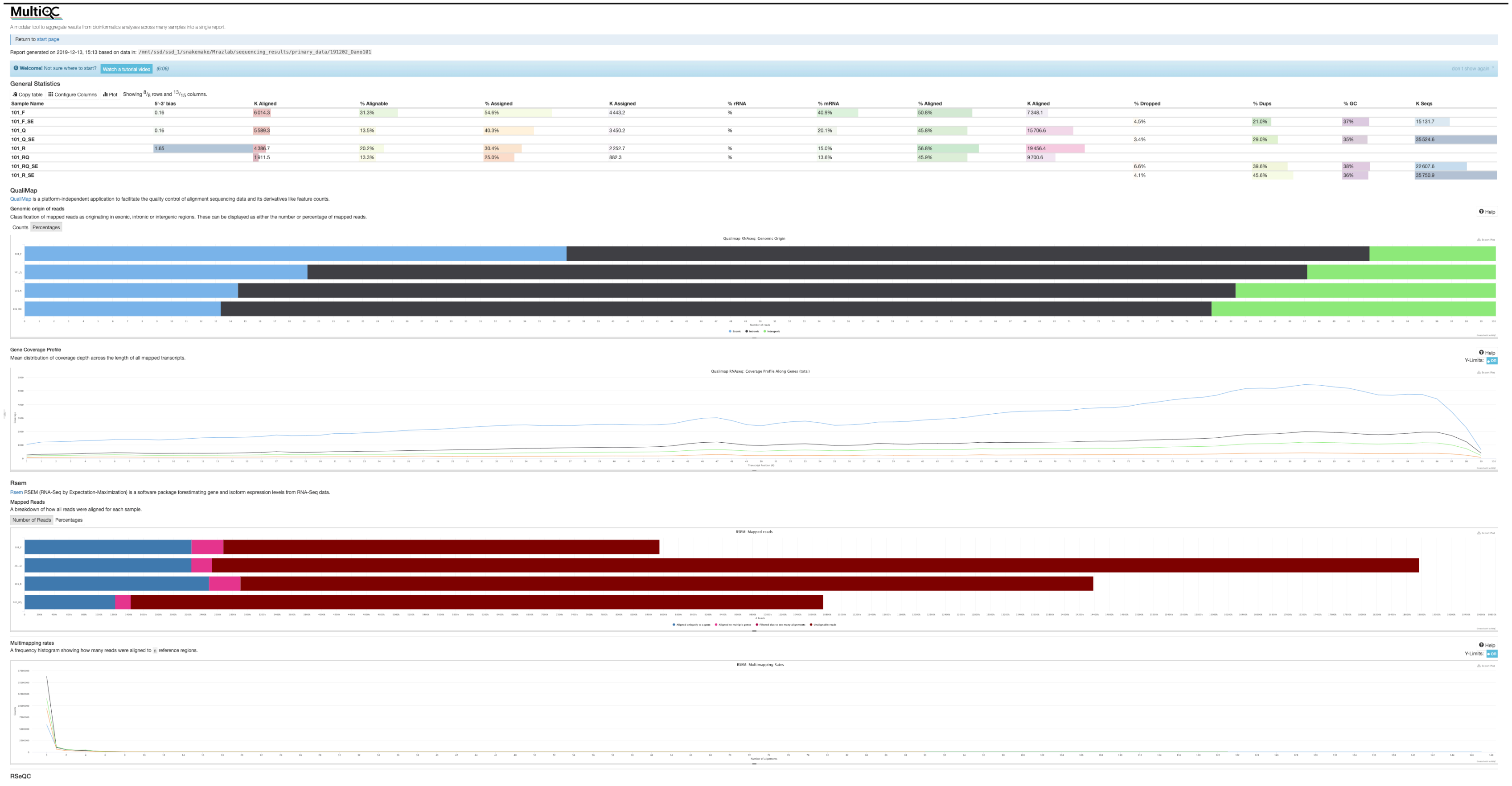
# Alignment - QC

- **Per gene coverage**
- **Variability of per gene mapping**
- **Gene counts distribution**
- **rRNA content estimate**
- **Tissue expression check - gtex**



# Alignment - QC

- **QC example – multiQC html**



MultiQC

A modular tool to aggregate results from bioinformatics analyses across many samples into a single report.

Return to start page

Report generated on 2019-12-13, 15:13 based on data in: `/mnt/ssd/ssd_1/snakemake/Mrazlab/sequencing_results/primary_data/191202_Dano101`

Welcome! Not sure where to start? Watch a tutorial video (6:06) don't show again

### General Statistics

Copy table | Configure Columns | Plot | Showing $^{8}/_{8}$ rows and $^{13}/_{15}$ columns.

| Sample Name | 5'-3' bias | K Aligned | % Alignable | % Assigned | K Assigned | % rRNA | % mRNA | % Aligned | K Aligned | % Dropped | % Dups | % GC | K Seqs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 101_F | 0.16 | 6 014.3 | 31.3% | 54.6% | 4 443.2 | % | 40.9% | 50.8% | 7 348.1 | | | | |
| 101_F_SE | | | | | | | | | | 4.5% | 21.0% | 37% | 15 131.7 |
| 101_Q | 0.16 | 5 589.3 | 13.5% | 40.3% | 3 450.2 | % | 20.1% | 45.8% | 15 706.6 | | | | |
| 101_Q_SE | | | | | | | | | | 3.4% | 29.0% | 35% | 35 524.6 |
| 101_R | 1.65 | 4 386.7 | 20.2% | 30.4% | 2 252.7 | % | 15.0% | 56.8% | 19 456.4 | | | | |
| 101_RQ | | 1 911.5 | 13.3% | 25.0% | 882.3 | % | 13.6% | 45.9% | 9 700.6 | | | | |
| 101_RQ_SE | | | | | | | | | | 6.6% | 39.6% | 38% | 22 607.6 |
| 101_R_SE | | | | | | | | | | 4.1% | 45.6% | 36% | 35 750.9 |

### QualiMap

QualiMap is a platform-independent application to facilitate the quality control of alignment sequencing data and its derivatives like feature counts.

**Genomic origin of reads**

Classification of mapped reads as originating in exonic, intronic or intergenic regions. These can be displayed as either the number or percentage of mapped reads.

**Gene Coverage Profile**

Mean distribution of coverage depth across the length of all mapped transcripts.

### Rsem

Rsem RSEM (RNA-Seq by Expectation-Maximization) is a software package forestimating gene and isoform expression levels from RNA-Seq data.

**Mapped Reads**

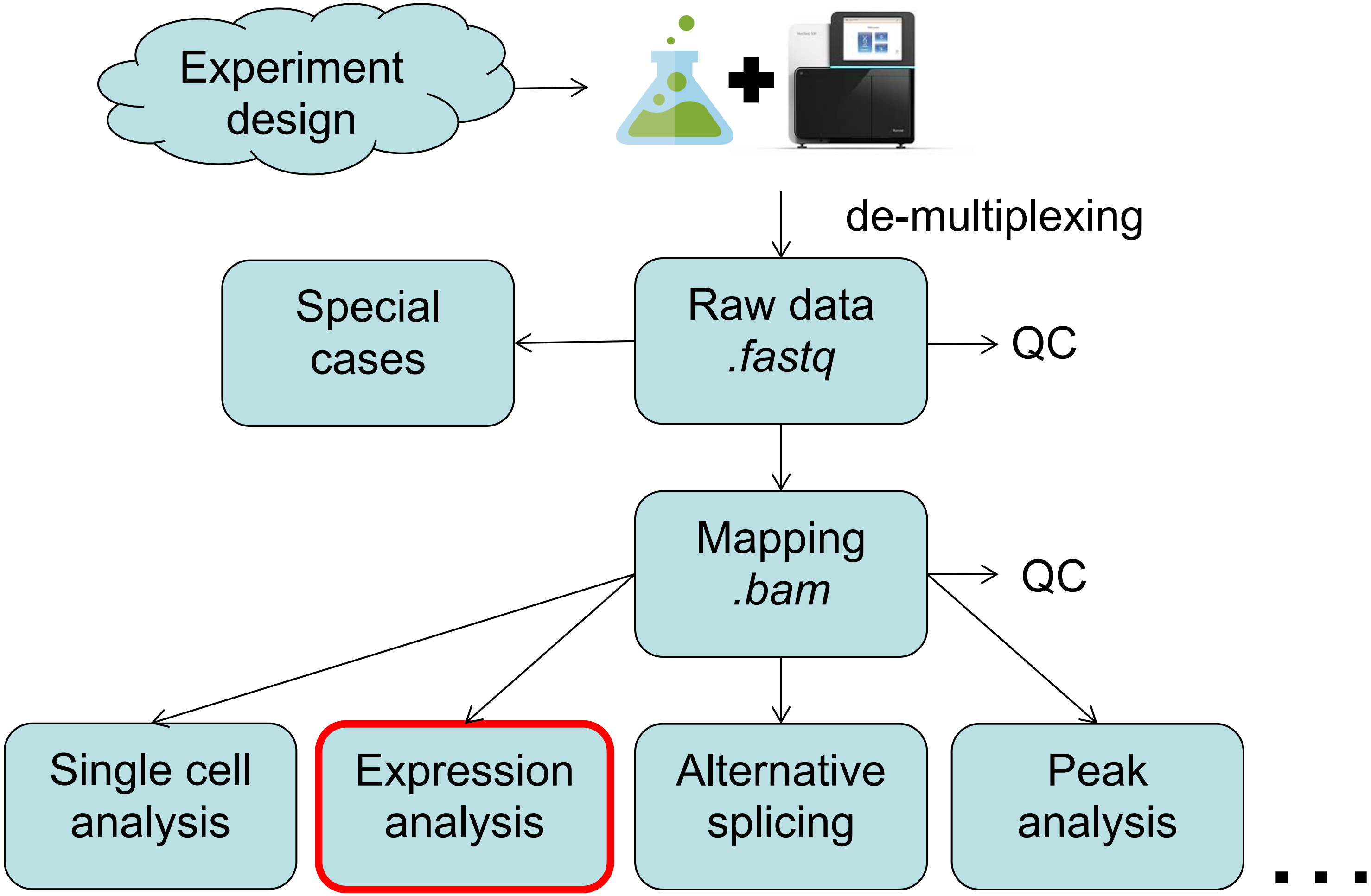
A breakdown of how all reads were aligned for each sample.

**Multimapping rates**

A frequency histogram showing how many reads were aligned to `n` reference regions.

### RSeQC

# NGS data analysis workflow



Experiment design

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QC

Mapping *.bam*

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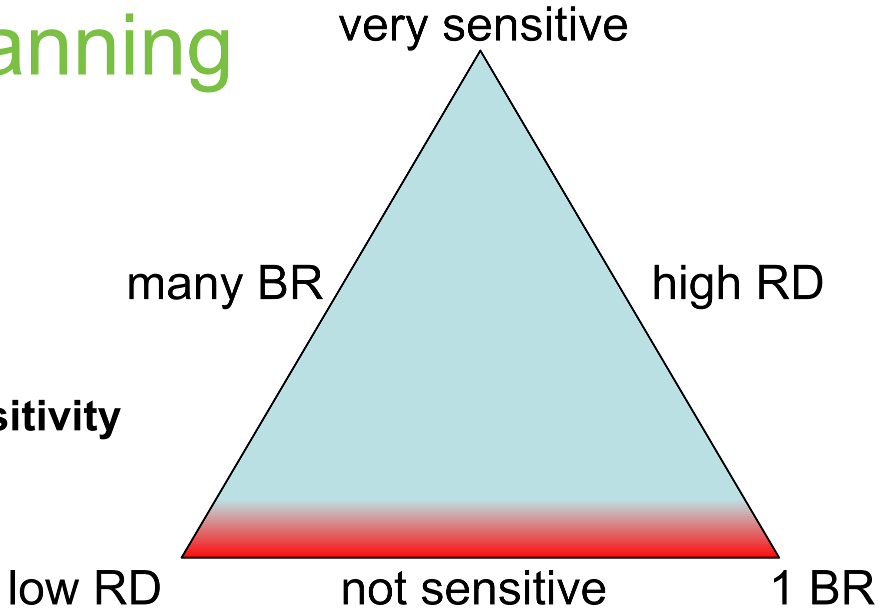
. . .

# Expression analysis - planning

- **3 way balance**
  - **Read depth**
  - **Biological replicates**
  - **Fold change (number of genes) sensitivity**



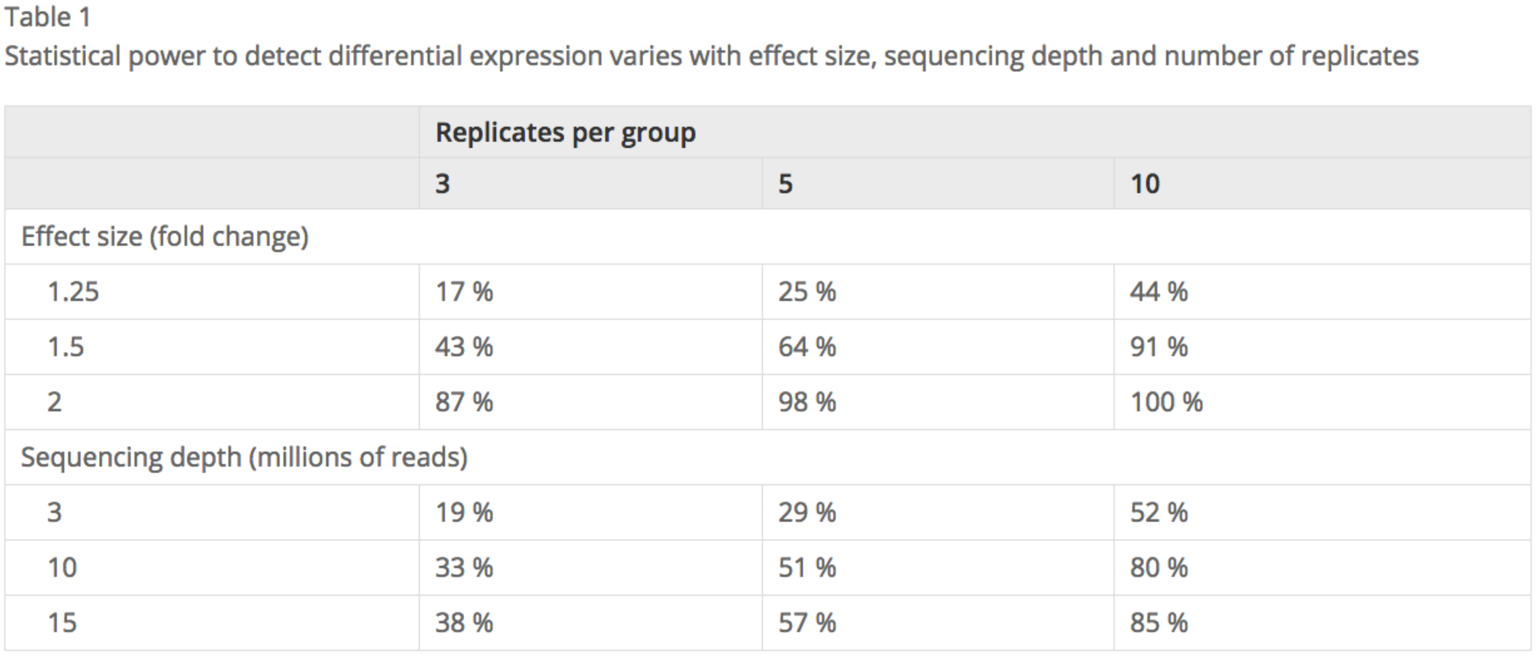


Table 1
Statistical power to detect differential expression varies with effect size, sequencing depth and number of replicates

| | Replicates per group | | |
|---|---|---|---|
| | **3** | **5** | **10** |
| Effect size (fold change) | | | |
| 1.25 | 17 % | 25 % | 44 % |
| 1.5 | 43 % | 64 % | 91 % |
| 2 | 87 % | 98 % | 100 % |
| Sequencing depth (millions of reads) | | | |
| 3 | 19 % | 29 % | 52 % |
| 10 | 33 % | 51 % | 80 % |
| 15 | 38 % | 57 % | 85 % |

# Expression analysis - planning

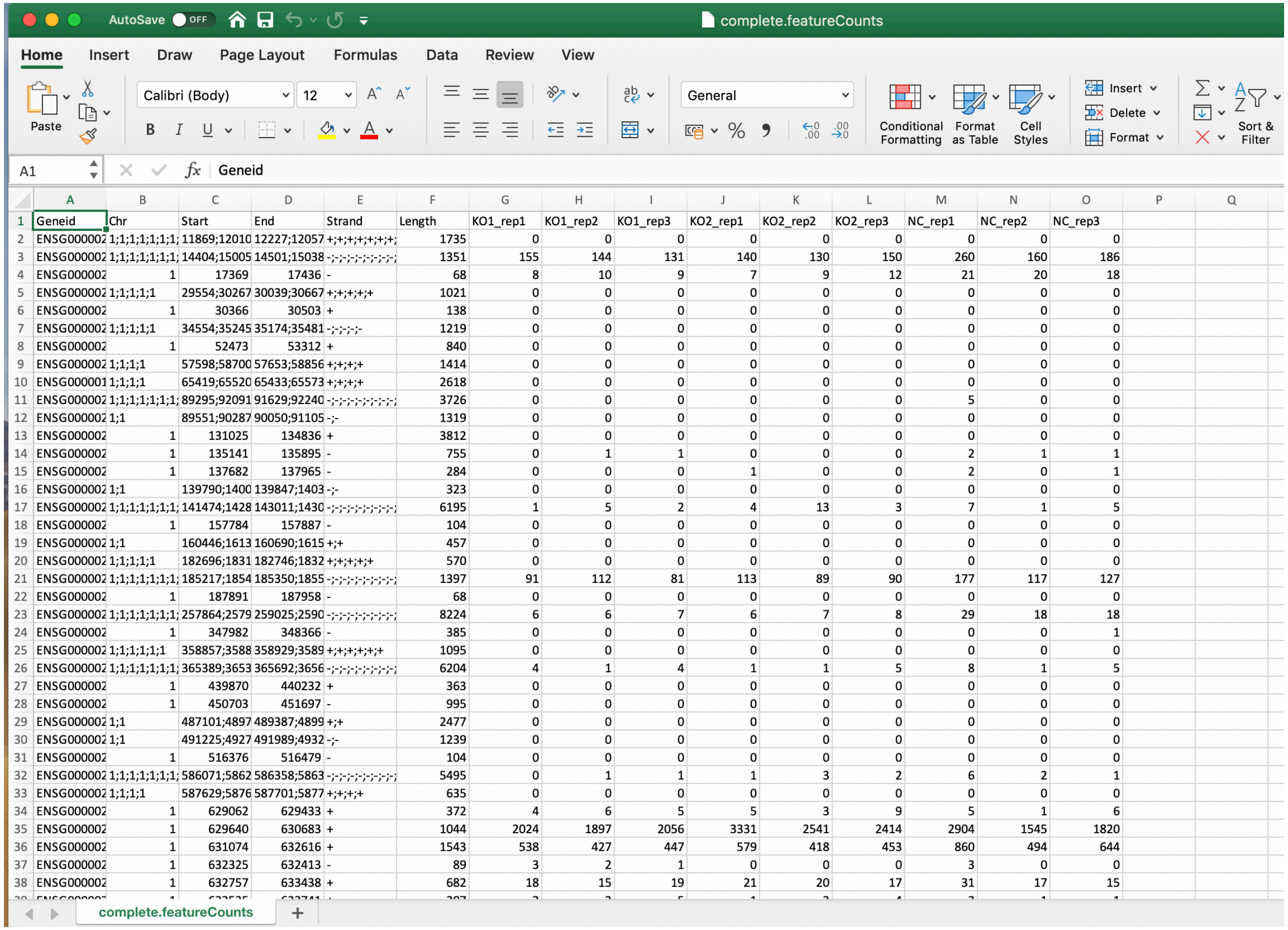
- **Depth**
- **Human ~ 22 000 genes = minimum 20 mil mapped reads**
- **Good 25 mil mapped reads**

- **Mapped reads!**
  - **rRNA removal**
  - **Size selection for sRNA**

- **Technical vs. biological**
  - **Technical only for technique testing**
- **Batch effect**
  - **Sample randomized sequencing**
- **Highly suggested minimum = 4 rep**

# Expression analysis

- **Raw counts**



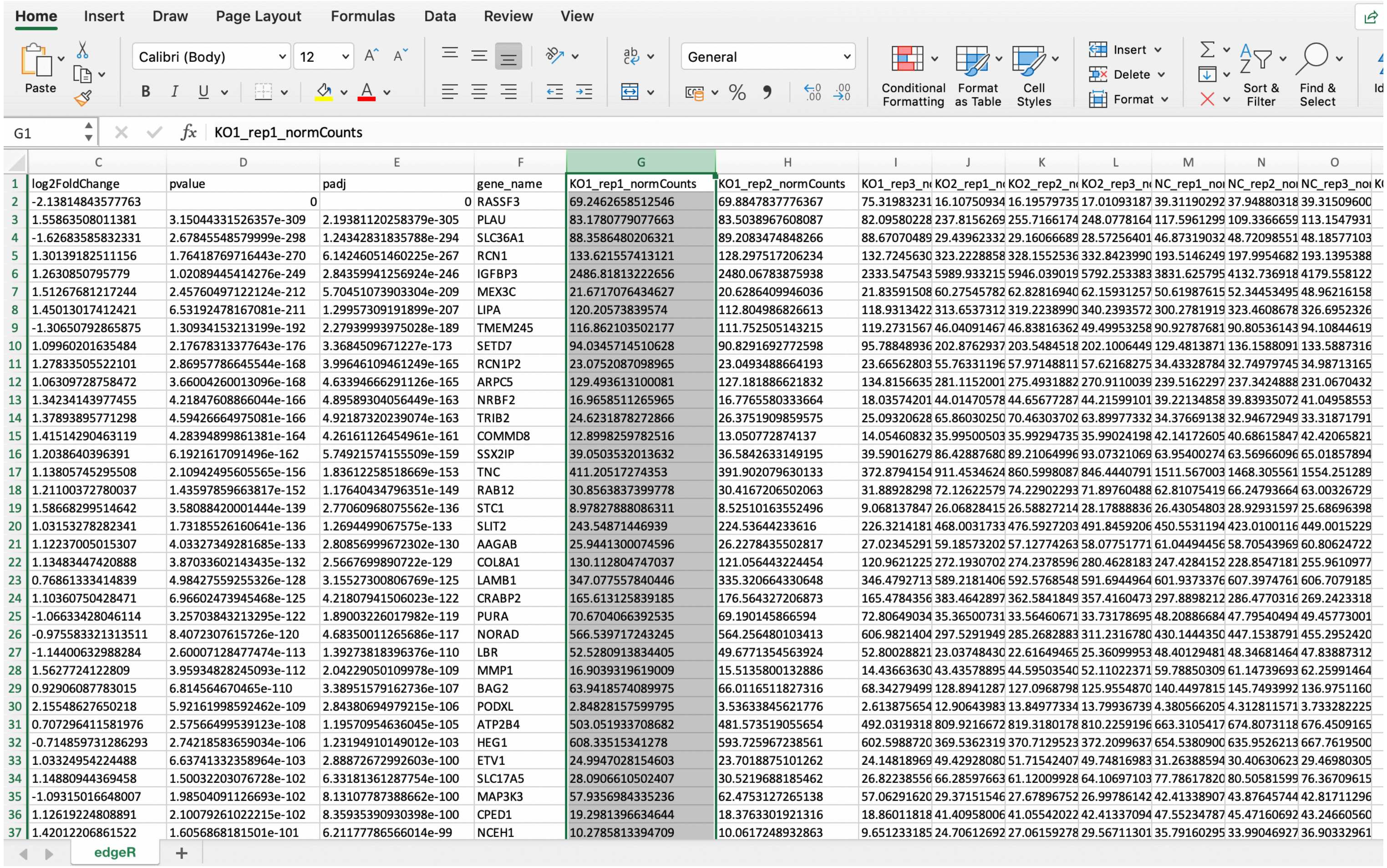
Geneid

| | A | B | C | D | E | F | G | H | I | J | K | L | M | N | O |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Geneid | Chr | Start | End | Strand | Length | KO1_rep1 | KO1_rep2 | KO1_rep3 | KO2_rep1 | KO2_rep2 | KO2_rep3 | NC_rep1 | NC_rep2 | NC_rep3 |
| 2 | ENSG000002 | 1;1;1;1;1;1;1; | 11869;12010 | 12227;12057 | +;+;+;+;+;+;+; | 1735 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | ENSG000002 | 1;1;1;1;1;1;1; | 14404;15005 | 14501;15038 | -;-;-;-;-;-;-;-; | 1351 | 155 | 144 | 131 | 140 | 130 | 150 | 260 | 160 | 186 |
| 4 | ENSG000002 | 1 | 17369 | 17436 | - | 68 | 8 | 10 | 9 | 7 | 9 | 12 | 21 | 20 | 18 |
| 5 | ENSG000002 | 1;1;1;1;1 | 29554;30267 | 30039;30667 | +;+;+;+;+ | 1021 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 6 | ENSG000002 | 1 | 30366 | 30503 | + | 138 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | ENSG000002 | 1;1;1;1;1 | 34554;35245 | 35174;35481 | -;-;-;-;- | 1219 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 8 | ENSG000002 | 1 | 52473 | 53312 | + | 840 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 9 | ENSG000002 | 1;1;1;1 | 57598;58700 | 57653;58856 | +;+;+;+ | 1414 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 10 | ENSG000001 | 1;1;1;1 | 65419;65520 | 65433;65573 | +;+;+;+ | 2618 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | ENSG000002 | 1;1;1;1;1;1;1; | 89295;92091 | 91629;92240 | -;-;-;-;-;-;-;-; | 3726 | 0 | 0 | 0 | 0 | 0 | 0 | 5 | 0 | 0 |
| 12 | ENSG000002 | 1;1 | 89551;90287 | 90050;91105 | -;- | 1319 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 13 | ENSG000002 | 1 | 131025 | 134836 | + | 3812 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 14 | ENSG000002 | 1 | 135141 | 135895 | - | 755 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 1 | 1 |
| 15 | ENSG000002 | 1 | 137682 | 137965 | - | 284 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 1 |
| 16 | ENSG000002 | 1;1 | 139790;1400 | 139847;1403 | -;- | 323 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 17 | ENSG000002 | 1;1;1;1;1;1;1; | 141474;1428 | 143011;1430 | -;-;-;-;-;-;-;-; | 6195 | 1 | 5 | 2 | 4 | 13 | 3 | 7 | 1 | 5 |
| 18 | ENSG000002 | 1 | 157784 | 157887 | - | 104 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 19 | ENSG000002 | 1;1 | 160446;1613 | 160690;1615 | +;+ | 457 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | ENSG000002 | 1;1;1;1;1 | 182696;1831 | 182746;1832 | +;+;+;+;+ | 570 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 21 | ENSG000002 | 1;1;1;1;1;1;1; | 185217;1854 | 185350;1855 | -;-;-;-;-;-;-;-; | 1397 | 91 | 112 | 81 | 113 | 89 | 90 | 177 | 117 | 127 |
| 22 | ENSG000002 | 1 | 187891 | 187958 | - | 68 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 23 | ENSG000002 | 1;1;1;1;1;1;1; | 257864;2579 | 259025;2590 | -;-;-;-;-;-;-;-; | 8224 | 6 | 6 | 7 | 6 | 7 | 8 | 29 | 18 | 18 |
| 24 | ENSG000002 | 1 | 347982 | 348366 | - | 385 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| 25 | ENSG000002 | 1;1;1;1;1;1 | 358857;3588 | 358929;3589 | +;+;+;+;+;+ | 1095 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 26 | ENSG000002 | 1;1;1;1;1;1;1; | 365389;3653 | 365692;3656 | -;-;-;-;-;-;-;-; | 6204 | 4 | 1 | 4 | 1 | 1 | 5 | 8 | 1 | 5 |
| 27 | ENSG000002 | 1 | 439870 | 440232 | + | 363 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 28 | ENSG000002 | 1 | 450703 | 451697 | - | 995 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 29 | ENSG000002 | 1;1 | 487101;4897 | 489387;4899 | +;+ | 2477 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 30 | ENSG000002 | 1;1 | 491225;4927 | 491989;4932 | -;- | 1239 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 31 | ENSG000002 | 1 | 516376 | 516479 | - | 104 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 32 | ENSG000002 | 1;1;1;1;1;1;1; | 586071;5862 | 586358;5863 | -;-;-;-;-;-;-;-; | 5495 | 0 | 1 | 1 | 1 | 3 | 2 | 6 | 2 | 1 |
| 33 | ENSG000002 | 1;1;1;1 | 587629;5876 | 587701;5877 | +;+;+;+ | 635 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 34 | ENSG000002 | 1 | 629062 | 629433 | + | 372 | 4 | 6 | 5 | 5 | 3 | 9 | 5 | 1 | 6 |
| 35 | ENSG000002 | 1 | 629640 | 630683 | + | 1044 | 2024 | 1897 | 2056 | 3331 | 2541 | 2414 | 2904 | 1545 | 1820 |
| 36 | ENSG000002 | 1 | 631074 | 632616 | + | 1543 | 538 | 427 | 447 | 579 | 418 | 453 | 860 | 494 | 644 |
| 37 | ENSG000002 | 1 | 632325 | 632413 | - | 89 | 3 | 2 | 1 | 0 | 0 | 0 | 3 | 0 | 0 |
| 38 | ENSG000002 | 1 | 632757 | 633438 | + | 682 | 18 | 15 | 19 | 21 | 20 | 17 | 31 | 17 | 15 |

complete.featureCounts

# Expression analysis

- **Result**



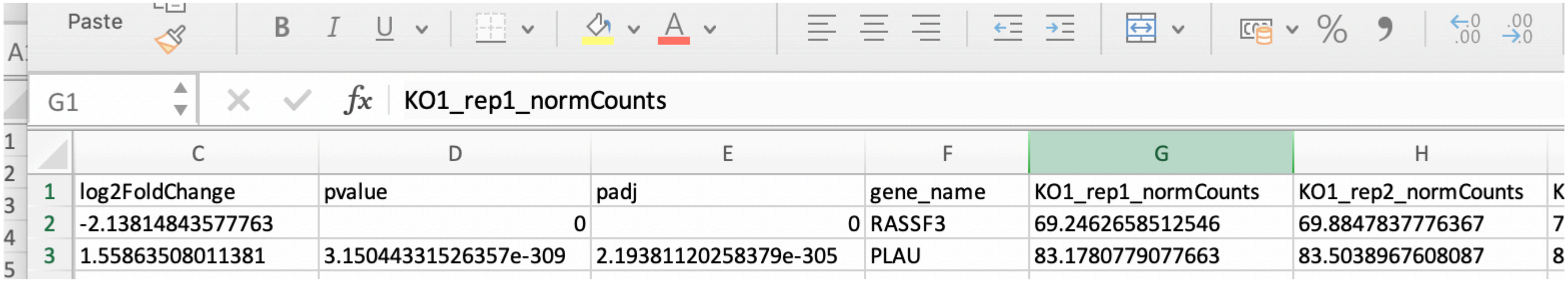
G1 fx KO1_rep1_normCounts

| | C | D | E | F | G | H | I | J | K | L | M | N | O | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | log2FoldChange | pvalue | padj | gene_name | KO1_rep1_normCounts | KO1_rep2_normCounts | KO1_rep3_nc | KO2_rep1_nc | KO2_rep2_nc | KO2_rep3_nc | NC_rep1_noi | NC_rep2_noi | NC_rep3_noi | K |
| 2 | -2.13814843577763 | 0 | 0 | RASSF3 | 69.2462658512546 | 69.8847837776367 | 75.31983231 | 16.10750934 | 16.19579735 | 17.01093187 | 39.31190292 | 37.94880318 | 39.31509600 | |
| 3 | 1.55863508011381 | 3.15044331526357e-309 | 2.19381120258379e-305 | PLAU | 83.1780779077663 | 83.5038967608087 | 82.09580228 | 237.8156269 | 255.7166174 | 248.0778164 | 117.5961299 | 109.3366659 | 113.1547931 | |
| 4 | -1.62683585832331 | 2.67845548579999e-298 | 1.24342831835788e-294 | SLC36A1 | 88.3586480206321 | 89.2083474848266 | 88.67070489 | 29.43962332 | 29.16066689 | 28.57256401 | 46.87319032 | 48.72098551 | 48.18577103 | |
| 5 | 1.30139182511156 | 1.76418769716443e-270 | 6.14246051460225e-267 | RCN1 | 133.621557413121 | 128.297517206234 | 132.7245630 | 323.2228858 | 328.1552536 | 332.8423990 | 193.5146249 | 197.9954682 | 193.1395388 | |
| 6 | 1.2630850795779 | 1.02089445414276e-249 | 2.84359941256924e-246 | IGFBP3 | 2486.8181322656 | 2480.06783875938 | 2333.547543 | 5989.933215 | 5946.039019 | 5792.253383 | 3831.625795 | 4132.736918 | 4179.558122 | |
| 7 | 1.51267681217244 | 2.45760497122124e-212 | 5.70451073903304e-209 | MEX3C | 21.6717076434627 | 20.6286409946036 | 21.83591508 | 60.27545782 | 62.82816940 | 62.15931257 | 50.61987615 | 52.34453495 | 48.96216158 | |
| 8 | 1.45013017412421 | 6.53192478167081e-211 | 1.29957309191899e-207 | LIPA | 120.20573839574 | 112.804986826613 | 118.9313422 | 313.6537312 | 319.2238990 | 340.2393572 | 300.2781919 | 323.4608678 | 326.6952326 | |
| 9 | -1.30650792865875 | 1.30934153213199e-192 | 2.27939993975028e-189 | TMEM245 | 116.862103502177 | 111.752505143215 | 119.2731567 | 46.04091467 | 46.83816362 | 49.49953258 | 90.92787681 | 90.80536143 | 94.10844619 | |
| 10 | 1.09960201635484 | 2.17678313377643e-176 | 3.3684509671227e-173 | SETD7 | 94.0345714510628 | 90.8291692772598 | 95.78848936 | 202.8762937 | 203.5484518 | 202.1006449 | 129.4813871 | 136.1588091 | 133.5887316 | |
| 11 | 1.27833505522101 | 2.86957786645544e-168 | 3.99646109461249e-165 | RCN1P2 | 23.0752087098965 | 23.0493488664193 | 23.66562803 | 55.76331196 | 57.97148811 | 57.62168275 | 34.43328784 | 32.74979745 | 34.98713165 | |
| 12 | 1.06309728758472 | 3.66004260013096e-168 | 4.63394666291126e-165 | ARPC5 | 129.49361310008 | 127.181886621832 | 134.8156635 | 281.1152001 | 275.4931882 | 270.9110039 | 239.5162297 | 237.3424888 | 231.0670432 | |
| 13 | 1.34234143977455 | 4.21847608866044e-166 | 4.89589304056449e-163 | NRBF2 | 16.9658511265965 | 16.7765580333664 | 18.03574201 | 44.01470578 | 44.65677287 | 44.21599101 | 39.22134858 | 39.83935072 | 41.04958553 | |
| 14 | 1.37893895771298 | 4.59426664975081e-166 | 4.92187320239074e-163 | TRIB2 | 24.6231878272866 | 26.3751909859575 | 25.09320628 | 65.86030250 | 70.46303702 | 63.89977332 | 34.37669138 | 32.94672949 | 33.31871791 | |
| 15 | 1.41514290463119 | 4.28394899861381e-164 | 4.26161126454961e-161 | COMMD8 | 12.8998259782516 | 13.050772874137 | 14.05460832 | 35.99500503 | 35.99294735 | 35.99024198 | 42.14172605 | 40.68615847 | 42.42065821 | |
| 16 | 1.2038640396391 | 6.1921617091496e-162 | 5.74921574155509e-159 | SSX2IP | 39.0503532013632 | 36.5842633149195 | 39.59016279 | 86.42887680 | 89.21064996 | 93.07321069 | 63.95400274 | 63.56966096 | 65.01857894 | |
| 17 | 1.13805745295508 | 2.10942495605565e-156 | 1.83612258518669e-153 | TNC | 411.2051727435 | 391.902079630133 | 372.8794154 | 911.4534624 | 860.5998087 | 846.4440791 | 1511.567003 | 1468.305561 | 1554.251289 | |
| 18 | 1.21100372780037 | 1.43597859663817e-152 | 1.17640434796351e-149 | RAB12 | 30.8563837399778 | 30.4167206502063 | 31.88928298 | 72.12622579 | 74.22902293 | 71.89760488 | 62.81075419 | 66.24793664 | 63.00326729 | |
| 19 | 1.58668299514642 | 3.58088420001444e-139 | 2.77060968075562e-136 | STC1 | 8.97827888086311 | 8.52510163552496 | 9.068137847 | 26.06828415 | 26.58827214 | 28.17888836 | 26.43054803 | 28.92931597 | 25.68696398 | |
| 20 | 1.03153278282341 | 1.73185526160641e-136 | 1.2694499067575e-133 | SLIT2 | 243.54871446939 | 224.53644233616 | 226.3214181 | 468.0031733 | 476.5927203 | 491.8459206 | 450.5531194 | 423.0100116 | 449.0015229 | |
| 21 | 1.12237005015307 | 4.03327349281685e-133 | 2.80856999672302e-130 | AAGAB | 25.9441300074596 | 26.2278435502817 | 27.02345291 | 59.18573202 | 57.12774263 | 58.07751771 | 61.04494456 | 58.70543969 | 60.80624722 | |
| 22 | 1.13483447420888 | 3.87033602143435e-132 | 2.5667699890722e-129 | COL8A1 | 130.112804747037 | 121.056443224454 | 120.9621225 | 272.1930702 | 274.2378596 | 280.4628183 | 247.4284152 | 228.8547181 | 255.9610977 | |
| 23 | 0.76861333414839 | 4.98427559255326e-128 | 3.15527300806769e-125 | LAMB1 | 347.077557840446 | 335.320664330648 | 346.4792713 | 589.2181406 | 592.5768548 | 591.6944964 | 601.9373376 | 607.3974761 | 606.7079185 | |
| 24 | 1.10360750428471 | 6.96602473945468e-125 | 4.21807941506023e-122 | CRABP2 | 165.613125839185 | 176.564327206873 | 165.4784356 | 383.4642897 | 362.5841849 | 357.4160473 | 297.8898212 | 286.4770316 | 269.2423318 | |
| 25 | -1.06633428046114 | 3.25703843213295e-122 | 1.89003226017982e-119 | PURA | 70.6704066392535 | 69.190145866594 | 72.80649034 | 35.36500731 | 33.56460671 | 33.73178695 | 48.20886684 | 47.79540494 | 49.45773001 | |
| 26 | -0.975583321313511 | 8.4072307615726e-120 | 4.68350011265686e-117 | NORAD | 566.539717243245 | 564.256480103413 | 606.9821404 | 297.5291949 | 285.2682883 | 311.2316780 | 430.1444350 | 447.1538791 | 455.2952420 | |
| 27 | -1.14400632988284 | 2.60007128477474e-113 | 1.39273818396376e-110 | LBR | 52.5280913834405 | 49.6771354563924 | 52.80028821 | 23.03748430 | 22.61649465 | 25.36099953 | 48.40129481 | 48.34681464 | 47.83887312 | |
| 28 | 1.5627724122809 | 3.95934828245093e-112 | 2.04229050109978e-109 | MMP1 | 16.9039319619009 | 15.5135800132886 | 14.43663630 | 43.43578895 | 44.59503540 | 52.11022371 | 59.78850309 | 61.14739693 | 62.25991464 | |
| 29 | 0.92906087783015 | 6.814564670465e-110 | 3.38951579162736e-107 | BAG2 | 63.941857408975 | 66.0116511827316 | 68.34279499 | 128.8941287 | 127.0968798 | 125.9554870 | 140.4497815 | 145.7493992 | 136.9751160 | |
| 30 | 2.15548627650218 | 5.92161998592462e-109 | 2.84380694979215e-106 | PODXL | 2.8482815759795 | 3.53633845621776 | 2.613875654 | 12.90643983 | 13.84977334 | 13.79936739 | 4.380566205 | 4.312811571 | 3.733282225 | |
| 31 | 0.707296411581976 | 2.57566499539123e-108 | 1.19570954636045e-105 | ATP2B4 | 503.051933708682 | 481.573519055654 | 492.0319318 | 809.9216672 | 819.3180178 | 810.2259196 | 663.3105417 | 674.8073118 | 676.4509165 | |
| 32 | -0.714859731286293 | 2.74218583659034e-106 | 1.23194910149012e-103 | HEG1 | 608.3351534127 | 593.725967238561 | 602.5988720 | 369.5362319 | 370.7129523 | 372.2099637 | 654.5380900 | 635.9526213 | 667.7619500 | |
| 33 | 1.03324954224488 | 6.63741332358964e-103 | 2.88872672992603e-100 | ETV1 | 24.9947028154603 | 23.7018875101262 | 24.14818969 | 49.42928080 | 51.71542407 | 49.74816983 | 31.26388594 | 30.40630623 | 29.46980305 | |
| 34 | 1.14880944369458 | 1.50032203076728e-102 | 6.33181361287754e-100 | SLC17A5 | 28.0906610502407 | 30.5219688185462 | 26.82238556 | 66.28597663 | 61.12009928 | 64.10697103 | 77.78617820 | 80.50581599 | 76.36709615 | |
| 35 | -1.09315016648007 | 1.98504091126693e-102 | 8.13107787388662e-100 | MAP3K3 | 57.9356984335236 | 62.475312726513 | 57.06291620 | 29.37151546 | 27.67896752 | 26.99786142 | 42.41338907 | 43.87645744 | 42.81711296 | |
| 36 | 1.12619224808891 | 2.10079261022215e-102 | 8.35935390930398e-100 | CPED1 | 19.2981396634644 | 18.3763301921316 | 18.86011818 | 41.40958006 | 41.05542022 | 42.41337094 | 47.55234787 | 45.47160692 | 43.24660560 | |
| 37 | 1.42012206861522 | 1.6056868181501e-101 | 6.21177786566014e-99 | NCEH1 | 10.2785813394709 | 10.0617248932863 | 9.651233185 | 24.70612692 | 27.06159278 | 29.56711301 | 35.79160295 | 33.99046927 | 36.90332961 | |

edgeR +

# Expression analysis

- **Result**
  - **normCounts**
    - **rpkm - Reads Per Kilobase of transcript per Million mapped reads**
    - **fpkm - Fragments Per Kilobase of transcript per Million mapped reads**
    - **tpm - Transcripts Per Million (TPM)**
      - **for every 1,000,000 RNA molecules in the RNA-seq sample, x came from this gene/transcript**
  - **log2FoldChange**
  - **pvalue**
  - **padj – pvalue adjusted for multiple testing**



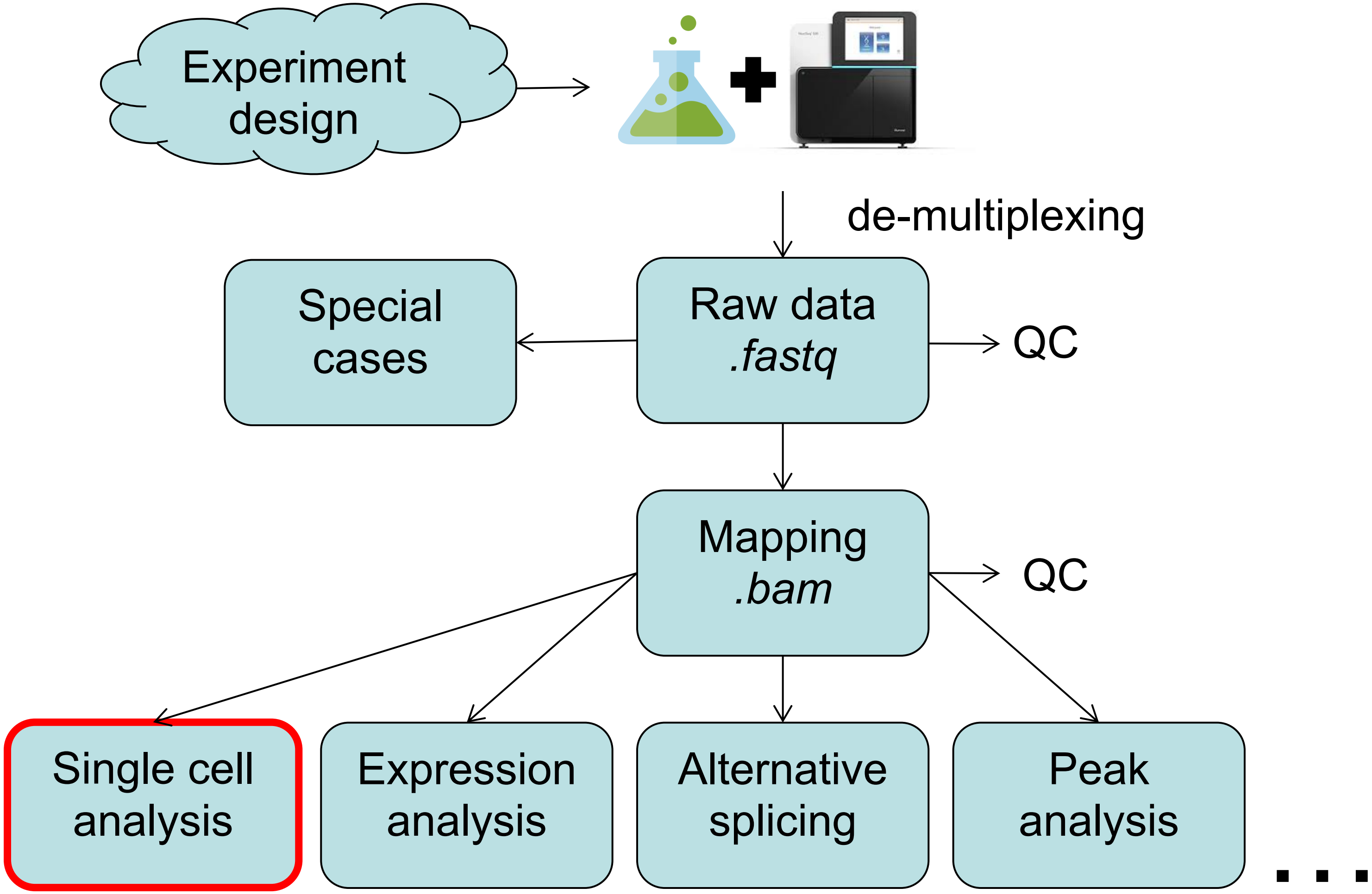
G1 fx KO1_rep1_normCounts

|  | C | D | E | F | G | H |  |
|---|---|---|---|---|---|---|---|
| 1 | log2FoldChange | pvalue | padj | gene_name | KO1_rep1_normCounts | KO1_rep2_normCounts | K |
| 2 | -2.13814843577763 | 0 | 0 | RASSF3 | 69.2462658512546 | 69.8847837776367 | 7 |
| 3 | 1.55863508011381 | 3.15044331526357e-309 | 2.19381120258379e-305 | PLAU | 83.1780779077663 | 83.5038967608087 | 8 |

# Expression analysis

- **Report example**

# NGS data analysis workflow



Experiment design

de-multiplexing

Special cases

Raw data *.fastq*

QC

Mapping *.bam*

QC

Single cell analysis

Expression analysis

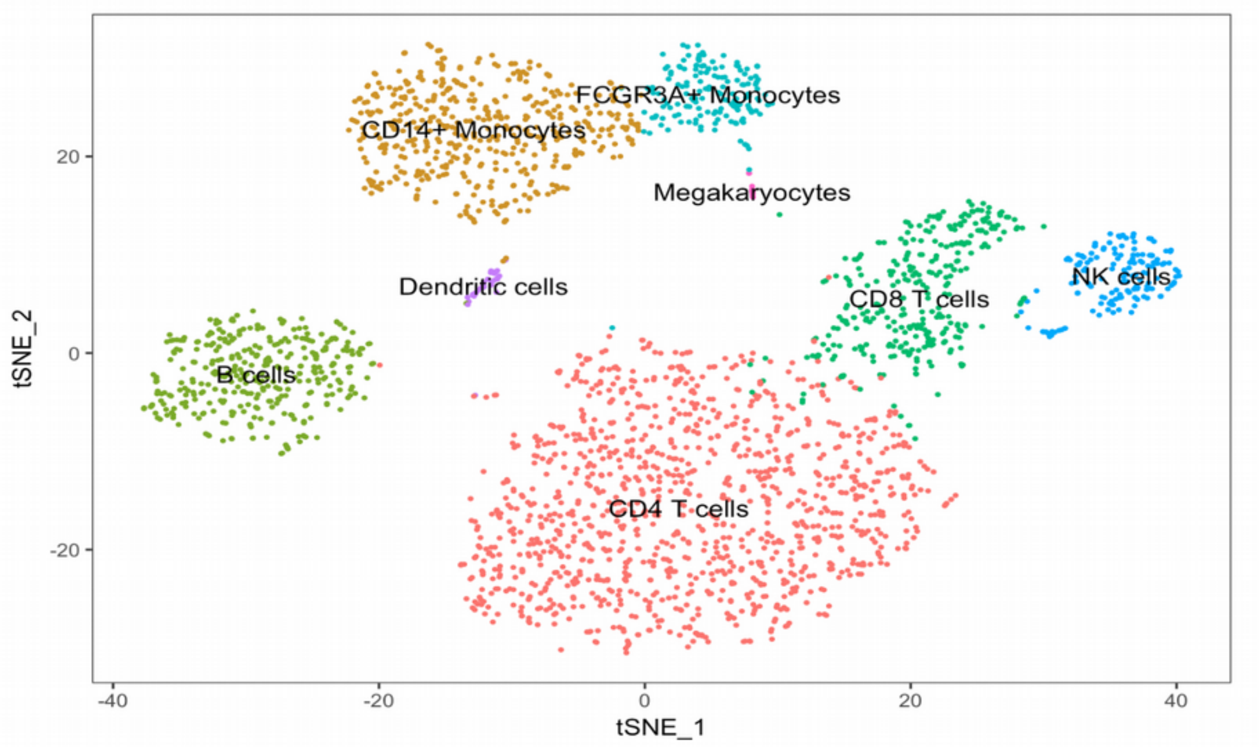
Alternative splicing

Peak analysis

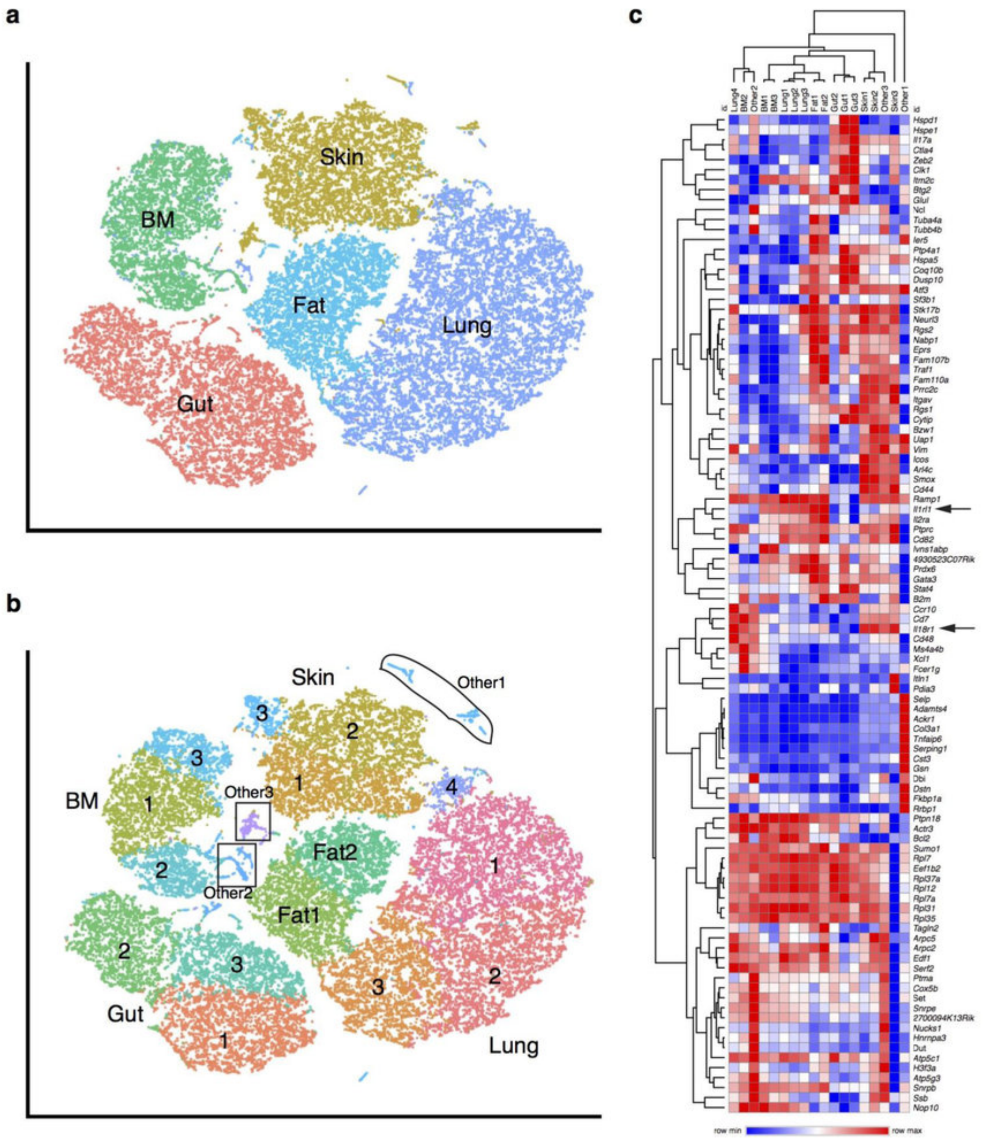
…

# Single cell analysis

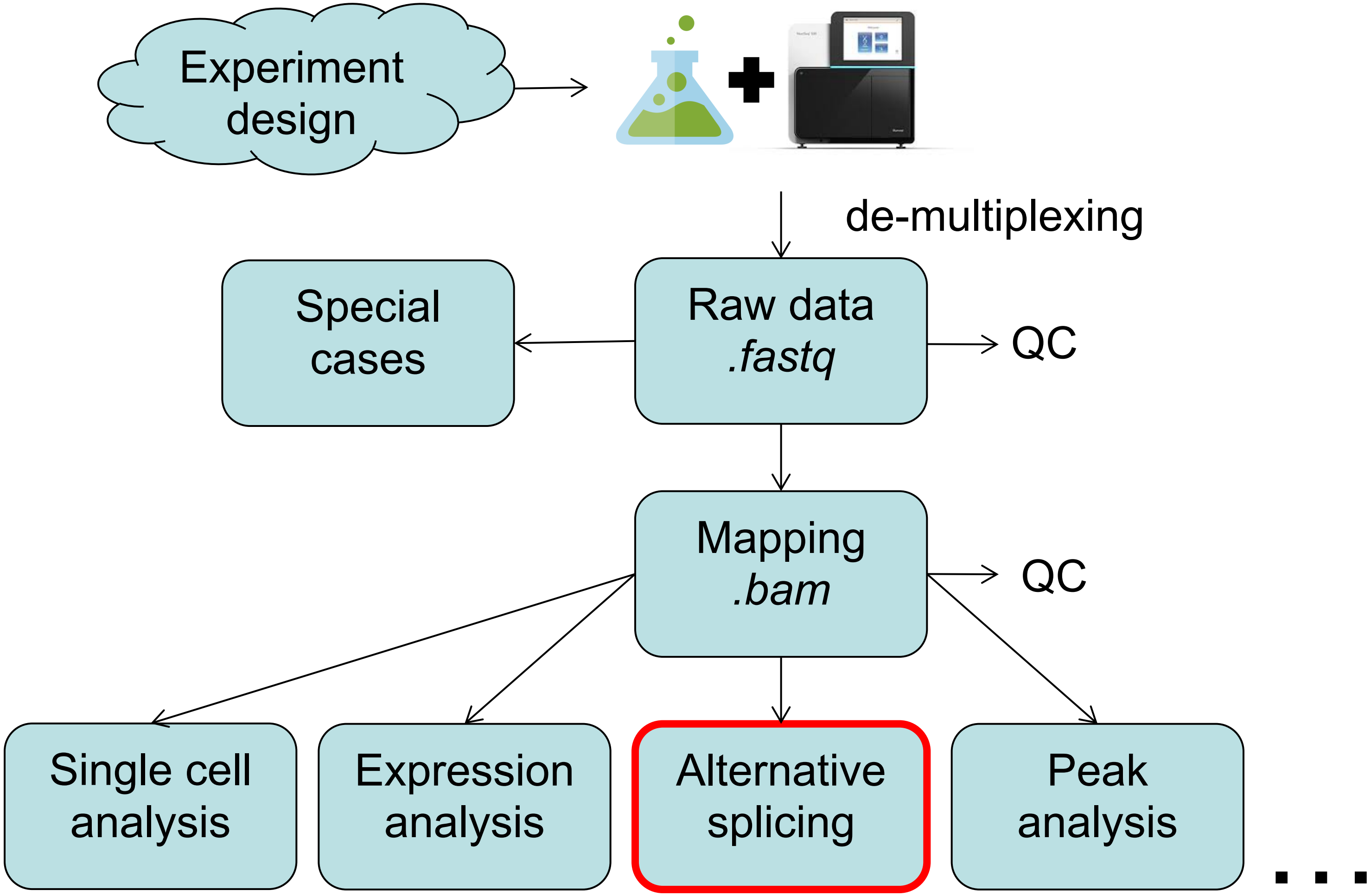
- **Cluster cells based on expression**
  - **Cleaning/Filtering step**
  - **Clustering**
  - **Dimension reduction**
    - **PCA**
    - **tSNE**
  - **Visualization**



# Single cell analysis



# NGS data analysis workflow



Experiment design

de-multiplexing

Special cases

Raw data *.fastq*

QC

Mapping *.bam*

QC

Single cell analysis

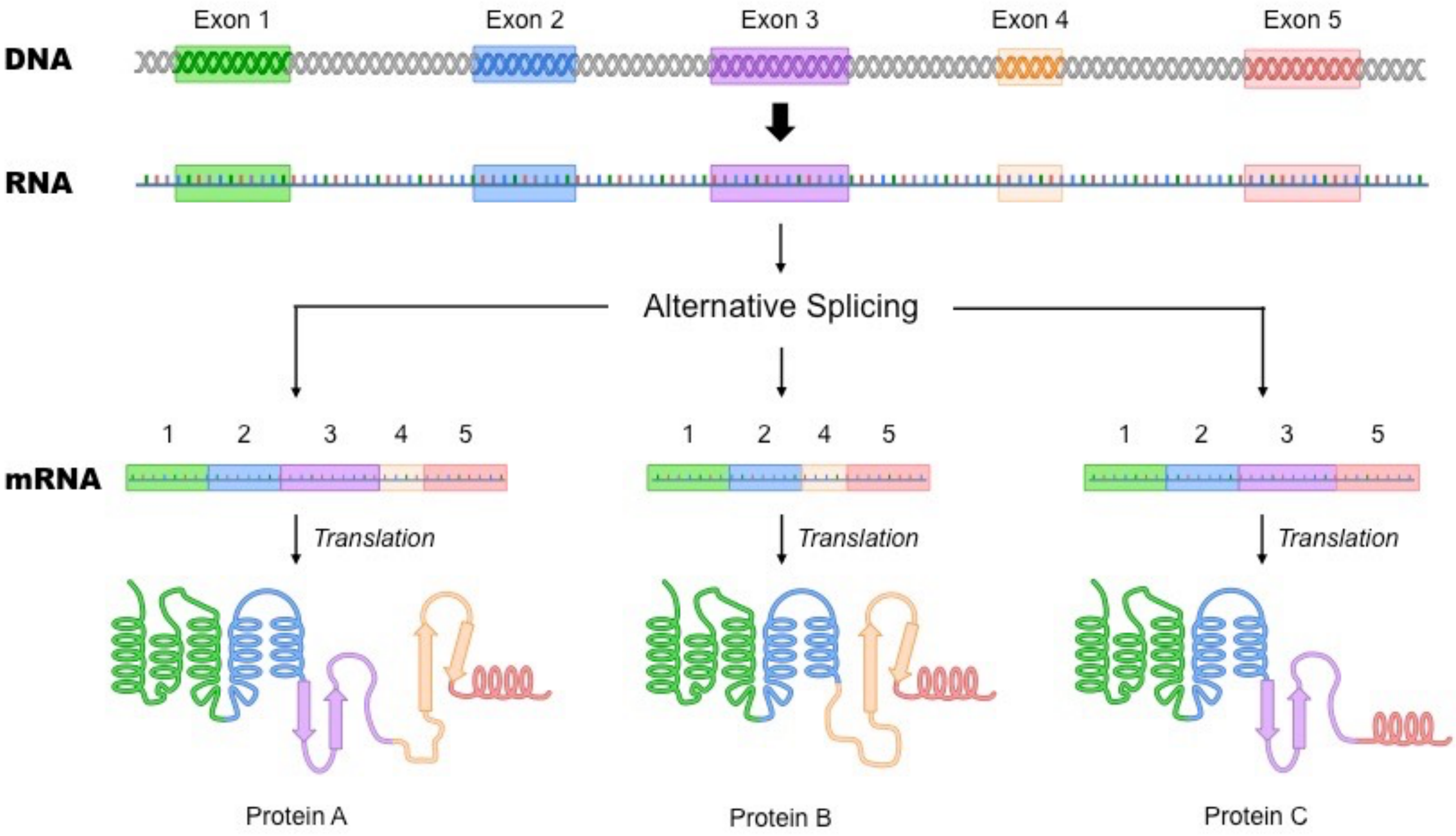
Expression analysis

Alternative splicing

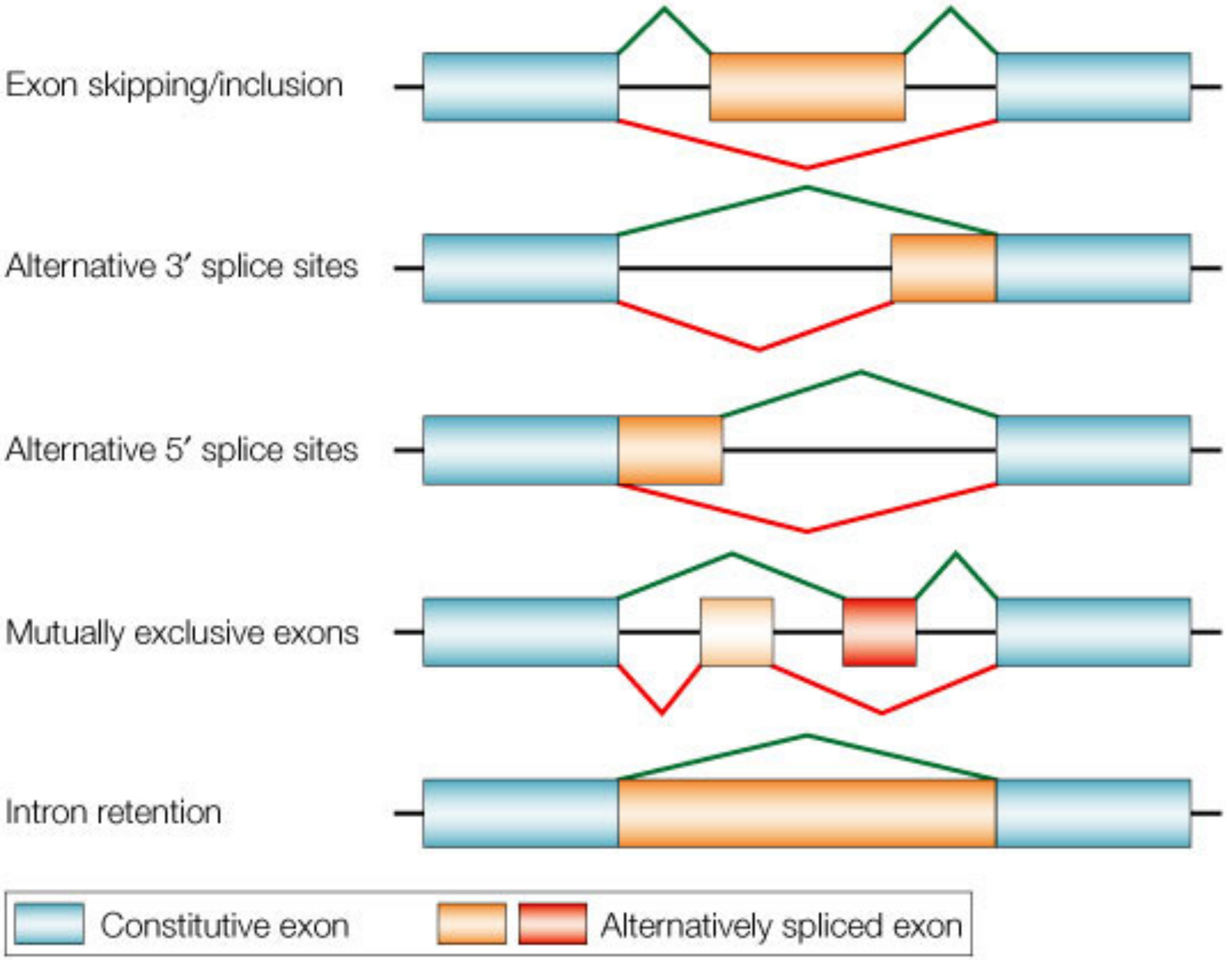
Peak analysis

…

# Alternative splicing



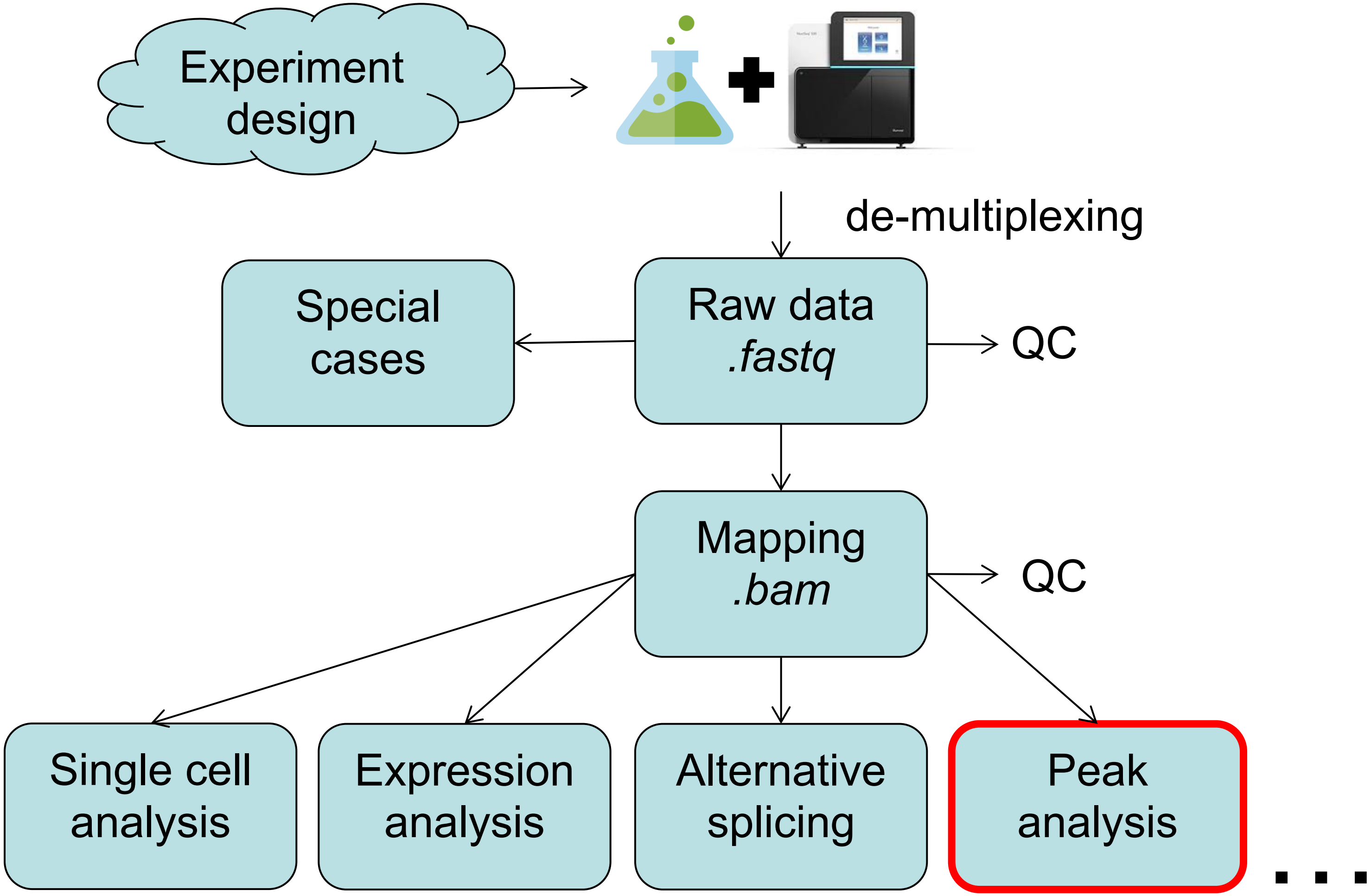
# Alternative splicing



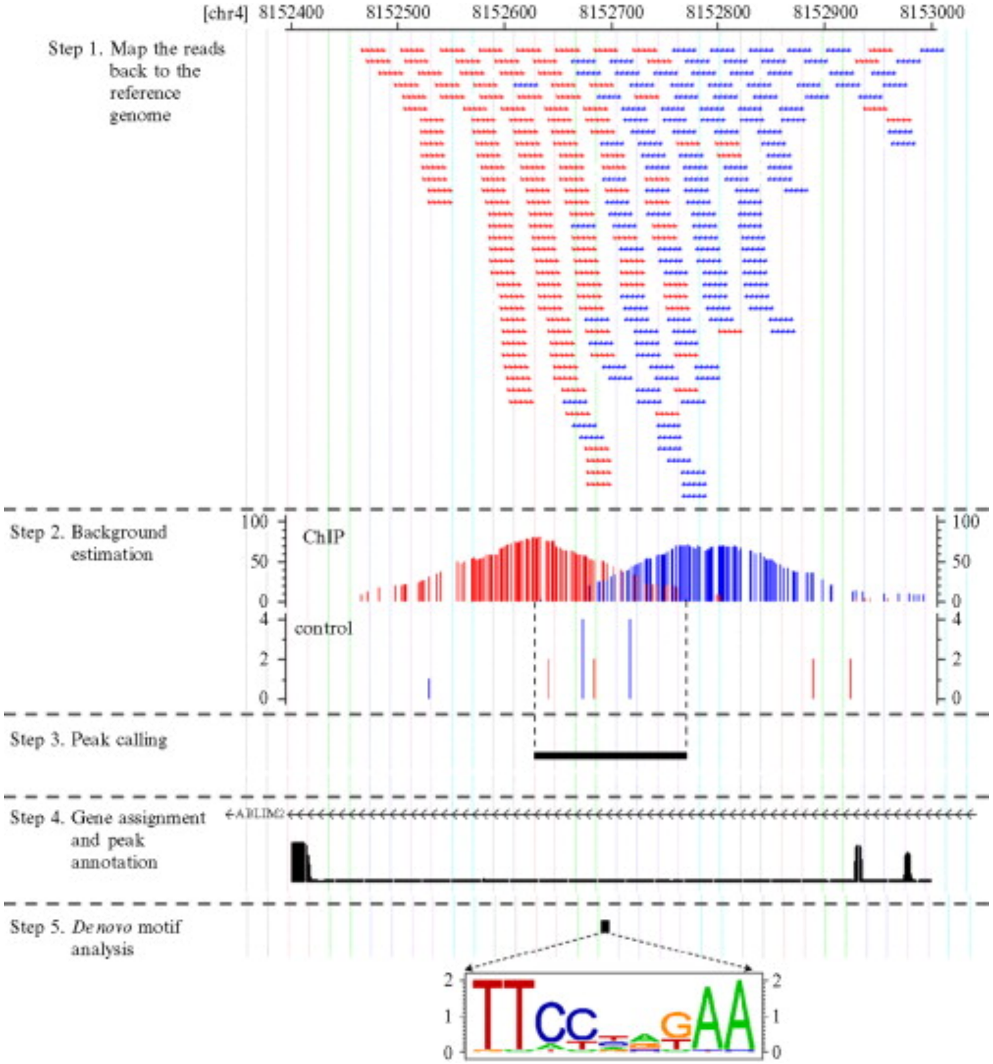
Exon skipping/inclusion
Alternative 3′ splice sites
Alternative 5′ splice sites
Mutually exclusive exons
Intron retention
Constitutive exon
Alternatively spliced exon

# Alternative splicing

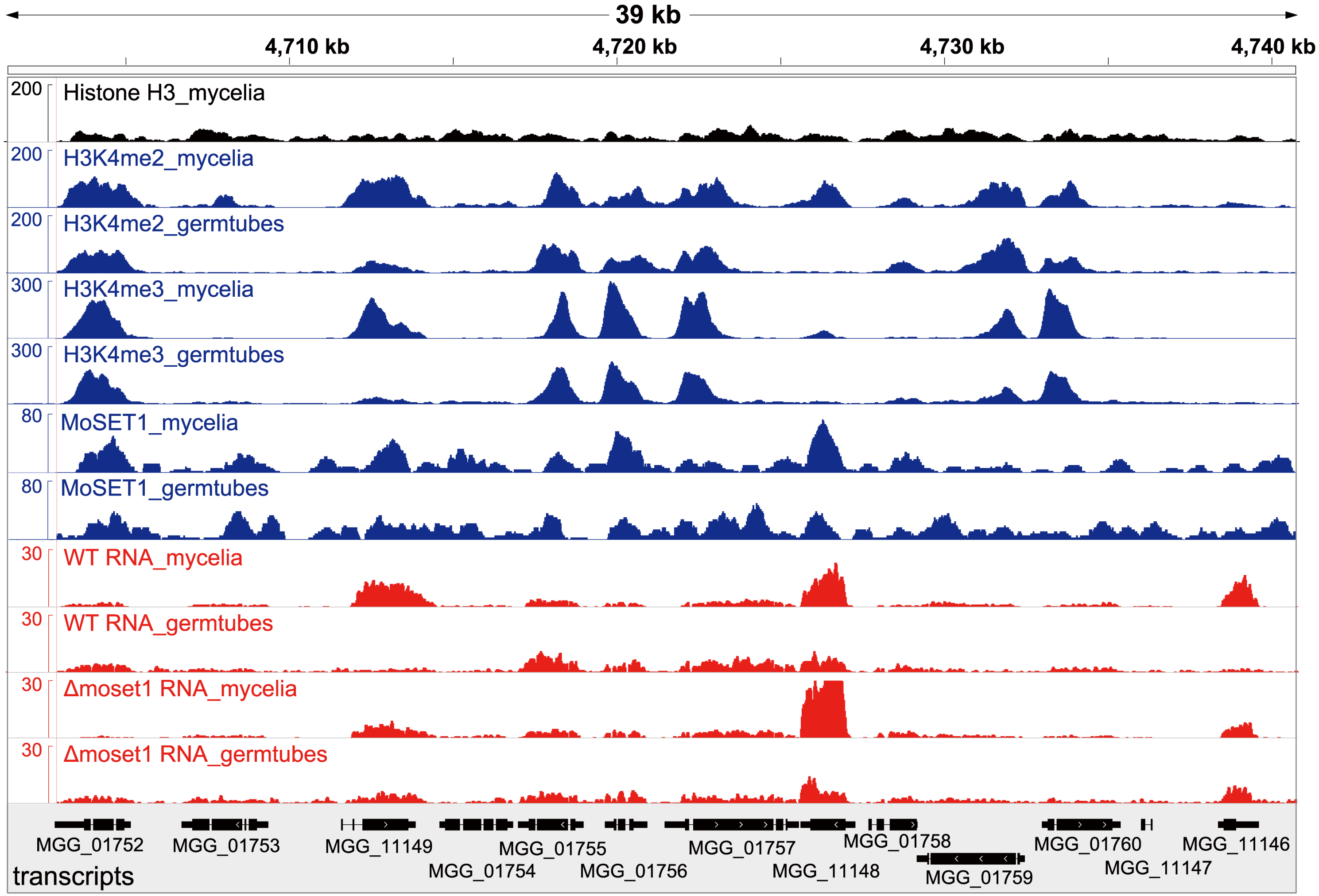
# NGS data analysis workflow



# Peak analysis


[chr4] 8152400 8152500 8152600 8152700 8152800 8152900 8153000
Step 1. Map the reads back to the reference genome
Step 2. Background estimation
100
50
0
ChIP
4
2
0
control
Step 3. Peak calling
Step 4. Gene assignment and peak annotation
←ABLIM2
Step 5. *De novo* motif analysis
2
1
0
TTCC GAA

# Peak analysis



# Thank you for your attention

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